Title:

Production of recombinant fragments of muscle acetylcholine receptor and their use for *ex vivo* immunoadsorption of anti-ACh receptor antibodies from myasthenic patients

CLAIMS

1. The procedure which consists of the production of a group of recombinant domains (large sequences of more than approximately 70 amino acids long; preferably about 200 amino acids long) of the human (or other primate) muscle nicotinic acetylcholine receptor (AChR) subunits alpha, beta, gamma, delta and epsilon, or mutant forms of said molecules (including substitutions of free cysteins by other amino acids and substitutions of the hydrophobic loops of the subunits corresponding to alpha128-142 by more hydrophilic sequences), or the alpha domain containing the P3A exon, the sum of which contains the major part of the extracellular domain of said receptor, and:
   a. each said subunit domain having the capacity (when permanently, preferably covalently, immobilized on insoluble carriers) of immunoadsorbing (eliminating) large fractions of anti-AChR antibodies from myasthenia gravis (MG) patients (for each subunit domain, these fractions exceed the 30% of the total anti-AChR antibodies in at least some MG sera, whereas the alpha subunit domain immunoadsorbs up to 94% of the anti-AChR antibodies from some MG sera.
   b. when said immunoadsorbents are used in combination or sequentially, their total absorbing capacity of MG antibodies, equals approximately the sum of the individually absorbed antibody fractions.
   Since each subunit domain eliminates the corresponding to said domain fraction of the total anti-AChR antibodies, the combined use of all or several of these domains is needed in order to eliminate the maximum possible amount of the anti-AChR antibodies from the patients.
2. The molecules of claim 1 (with the characteristics 1a and 1b), which consist of the extracellular N-terminal parts of the human AChR subunits alpha, beta, gamma, delta and epsilon or mutant forms of said molecules.
3. The procedure that consists of the permanent (covalent) immobilization of the molecules of claims 1 and 2 on insoluble carriers, selected from several suitable matrixes known in the art including agaroses (for example CNBr-Sepharose), celluloses, porous glass, silica, resins, synthetic matrixes including acrylamide derivatives, methacrylamide derivatives or polystyrene derivatives etc, in various forms including beads, fibrous form, sheets or hollow fibers, with spacer arms or without, by approaches known in the art for immobilization of other ligands.
4. The procedure which consists of using the permanently immobilized molecules of claim 3, separately and in combination (i.e. several or all of them together or sequentially), for *in vitro* elimination of anti-AChR antibodies (preferably the majority) from sera of MG patients.
5. The use of the permanently immobilized molecules of claim 3, preferably in combination, and of the application of the procedure of claim 4, for therapeutic *ex vivo* elimination/apheresis of the majority of the anti-AChR antibodies from the blood of MG patients.

6. The procedure which consists of the production of the molecules of claim 1 (with the characteristics 1a and 1b), expressed in eukaryotic expression systems (like yeast *Pichia pastoris,* Semliki Forest Virus system, Baculovirus system) expressed separately or coexpressed.
7. The molecules of claim 6, which consist of the extracellular N-terminal parts of the human AChR subunits alpha, beta, gamma, delta and epsilon, or mutant forms of said molecules.
8. The procedure which consists of the permanent (covalent) immobilization of the molecules of claims 6 and 7 on insoluble carriers as those in claim 3.
9. The procedure which consists of using the permanently immobilized molecules of claim 8, separately and in combination (i.e. several or all of them together or sequentially), for *in vitro* elimination of antibodies (preferably the majority of them) against human muscle AChR, from sera of MG patients.
10. The use of the permanently immobilized molecules of claim 8, preferably in combination (together or sequentially), and of the application of the procedure of claim 9, for therapeutic *ex vivo* elimination/apheresis of the majority of the anti-AChR antibodies from the blood of MG patients.